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RESEARCH

Eco-toxicological effects assessment: comparative characteristics of environmental conditions and status of vertebrate indicator species in the "Dnepr" launch vehicle accident zone

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Abstract Space rockets are regularly launched from the Baikonur Cosmodrome in Kazakhstan. The ecological impact of territories affected by the consequences of space rocket activities on the environment is a significant concern. On July 27, 2006, the Dnepr rocket was launched from the Baikonur Cosmodrome, resulting in an abnormal situation and its emergency fall. The rocket fuel components, heptyl, and amyl, led to decontamination and reclamation activities. However, the self-restoration of soil and vegetation cover is currently in the earliest stages. Consequently, an assessment of the remote consequences of the accidental fall of the Dnepr launch vehicle for environmental objects was conducted. The study involved eco-toxicological characterization of habitat conditions, assessment of species diversity, determination of key morphophenetic indicators, and the genetic status of indicator species as the main parameters. The results revealed an increased level of genotoxicity in soils at the accident site. While there were deviations from the norm in some morphophenetic and cytogenetic parameters in indicator animals, they were not critical for their existence in this territory. Possibly, at the physiological level, they exhibit a high enough adaptation potential and compensatory mechanism.

Keywords Indicator animals · Micronuclei · Rocket and space activities · Allium test · Chromosomal aberrations

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Introduction

The launch operations infrastructure of the Baikonur Cosmodrome has some degree of risk to the environment and the population in the places where boosters are being dropped (Dallas et al., 2020; Koroleva et al., 2018), notably, it is in certain areas of the Kyzylorda region. To ensure the safety of the launch sites at the cosmodrome, it is mandatory to provide ecological measures for launch vehicle blastoff based on a wide-ranging set of activities to be gained under

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the intergovernmental agreement between Kazakhstan and Russia. The treaty obligation is committed to assess and minimize the degree of possible negative environmental impact on the region during the regular launch and the full-scale test of the first stage. At the same time, despite the planning and acts performed according to a contractual arrangement, the risk of an emergency and problems of serious environmental concern under an abnormal launch cannot be completely excluded. The very same occurred on July 27, 2006, during another launch of the Dnepr rocket designed for delivery of a group of satellites. The ensuing investigation revealed that the accident led to an emergency termination of the flight and a failure of the launch vehicle (Koroleva et al., 2021; Bisarieva et al., 2012).

The search team identified the fall site of the 1st and 2nd stages of the Dnepr rocket, located in the northern part of the Kyzylkum desert, 130 km south of the Baikonur Cosmodrome (coordinates of the center of the fall: N 44.34247, E 62.58139). The examination showed that at the site of the launch vehicle, there was an explosion of rocket fuel components (RFC—heptyl and amyl), which pose the greatest danger to humans and other biological organisms as they have an extremely toxic and mutagenic effect (Hu et al., 2022; Kenessov et al., 2012; Koroleva et al., 2023; Lovinskaya et al., 2017). As a result of the explosion, the so-called "Big Crater" was formed with a diameter of 50 m, a depth of 15 m, and a parapet height of 2.5 m (Fig. 1). Large fragments of Dnepr rocket stages were discovered at a distance of approximately 400–500 m from the center of the crater. Individual small fragments were scattered at a distance of up to 800–900 m. The calculation method showed that the blast wave lifted into the air a clay mass weighing about 90 thousand tons, which was mixed with RFC and their decay products. Part of this mass of ancient alluvial loamy deposits and saline tertiary clays formed a parapet, and most of it dispersed in the southeastern and eastern directions, at a distance of 500 m—3–5 km (Bisarieva et al., 2012).

A study of the explosion and a crash site of the Dnepr showed that it is located in an area with rare *Haloxylon persicum—Anabasis salsa—Salsola orientalis* vegetation assemblages. The soil cover is represented by takyr and takyr-like varieties of soil. In terms of its physic-chemical properties, it is densely structured with low water permeability and a fairly high absorption capacity, so that it can retain pollutants and water-soluble contaminants in the upper layer. Immediately after the accident, a soil study was carried out to determine the content of rocket fuel components. Analysis of soil samples taken from the area showed a multiple excess of the MPL (maximum permissible level) of heptyl—UDMH (unsymmetrical dimethylhydrazine, more precisely 1,1-dimethylhydrazine), and in the center of the "Big Crater"—up to 228 MPL. MPL of heptyl in soil 0.1 mg/kg (Nauryzbaev, 2017). To eliminate RFC contamination and restore the relief according to the agreed regulations, detoxification and reclamation measures were attempted in this area (Fig. 1) (Koroleva et al., 2021).

Fig. 1 The Big Crater, May 2007

However, the natural regeneration of ecosystems from such disasters takes a long time (Lednev et al., 2019) and recent observations showed that despite extended periods natural land cover has not recovered from the accident. This is confirmed by the presence of the surface transformation in the form of wind and water erosion, as well as the surviving traces of vortex planning and road degradation (Fig. 2).

Detectable degradation elements in the base segment of environmental objects (soil, vegetation) clearly indicate the long-term effects of the Dnepr launch vehicle (LV) accident. It should be noted that the location of the LV fall is an extremely fragile natural occurring desert region (Kyzylkum) which is unstable to negative impacts. According to Morris et al. (2011), restoration of the vegetation of this natural area after impact can take up to 100 years. At present, the process of restoration of natural vegetation

Fig. 2 Reclamated section of the "Big Crater," May 2023

in the area of the Dnieper LV crash site is growing, albeit slowly (only 28 species in 11 years). The total species list of higher vascular plants recorded throughout the study period in the vicinity of Big Crater comprises 197 species from 33 families distributed among 121 genera (Atygayev et al., 2021; Veselova et al., 2022). In addition to the chemical and physical factors of the accident, the results of the impact on environmental objects may include undertaken measures aimed at reclamation and detoxification. Most of the published literature on this issue is devoted to the impact of rocket launches from the Baikonur Cosmodrome on the terrestrial ecosystems of the Republic of Kazakhstan and the Russian Federation (Lednev et al., 2018; Krechetov et al., 2011; Lednev et al., 2020a; Maikanov et al., 2022). However, these studies mainly consider the state of soil and vegetation cover without analyzing its genotoxicity and the impact on the population health in the region (Sidorov et al., 2006; Carlsen et al., 2010; Semenova, 2016; Askarov et al., 2022). The state and genetic status of the fauna in the crash sites of the launch vehicle and territories around are less investigated (Xue et al., 2021).

This implies that a more detailed study of the long-term environmental consequences of a launch vehicle accident is required, especially with regard to animals, as the final link of the ecosystem. In this case, in order to study the condition of vertebrate animals living in the territory exposed to RFC contamination, first and foremost is to determine the indicators of mutagenicity and genotoxicity of soils. In this context, the establishment of modern toxicological characteristics of soils from the site of the Dnepr launch vehicle accident can be studied using special test systems (Bianchi et al., 2016; Leme and Marin-Morales, 2009; Fenech et al., 2011; Guilherme et al., 2008; Poletta et al., 2013).

Desert mammal and reptile species are suitable indicator groups among vertebrate animals for biological indication to determine the remote consequences of the accident under the existing natural and climatic conditions(Carlsen et al., 2010; Semenova, 2016; Askarov et al., 2022; Xue et al., 2021; Bianchi et al., 2016; Leme and Marin-Morales, 2009; Fenech et al., 2011; Guilherme et al., 2008; Poletta et al., 2013; Poletta et al., 2017; Poletta and Siroski, 2019; Leung et al., 2022; Zakharov, 2001; Pollo, 2015; Leme and Marin-Morales, 2009). Mammals, especially rodents, are traditionally used to study the genotoxicity of various substances and anthropogenic factors in laboratory conditions and as bioindicators (Mitkovska et al., 2012; Şekeroğlu et al., 2013; Lovinskaya et al., 2017). Reptiles are also sensitive bioindicators of environmental pollution (Poletta and Siroski, 2019). Depending on the need to assess a particular habitat, different species of reptiles are used, including snakes, crocodiles (González et al., 2021), turtles (Castaño et al., 2020), and various species of lizards (Simonyan et al., 2018; Schaumburg et al., 2012). This is particularly relevant when analyzing the impact of anthropogenic factors in desert and semi-desert regions, where the choice of indicator animals is very limited (Silva et al., 2021), as in our case.

Assessing species diversity and examining the characteristics of their habitat, a complex of morphometric and cytogenetic methods is employed to evaluate the state of populations of indicator

species. These methods are integral components of the main parameters used in the study of animals. This kind of combination to determine the basic ecological, exterior, and interior characteristics of mammals, as well as the fluctuating asymmetry of bilateral characteristics of reptiles are the most simple but most sensitive signs of developmental stability and the state of populations of different animal species (Poletta et al., 2017; Poletta and Siroski, 2019; Leung et al., 2022; Zakharov, 2001). Also, one of the indicators of the state of the body is cytogenetic homeostasis, manifested in the maintenance of the karyotype (Pollo, 2015). In many cases, the easiest way to identify homeostasis is using the micronucleus test—a method for determining the genotoxicity of various agents and environmental factors. It involves determining the frequency of appearance of micronuclei in populations of interphase cells. At present, cytometry analysis is a tool conducted for micronucleus analysis in nucleated cells, which includes the analysis of a wide range of morphological types of nuclear abnormalities.

Hence, based on the retrospective review of the post-emergency situation with elements of negative environmental manifestations detailed environmental monitoring is required to study its long-term consequences in the area where the Dnepr launch vehicle crashed. The integrated research involves a comparative examination of eco-toxicological characteristics of living conditions and assessment of the indicator species state (namely mammals and reptiles) combined with the determination of morphophenetic parameters of animals and their genetic status through the micronucleus test.

Materials and methods

This study was approved by the Local Ethical Commission of the Institute of Human and Animal Physiology (Almaty, Kazakhstan; approval # 3 by 28.03. 2022г).

The crash site of the Dnepr LV is located in the Karmaksha district of Kyzylorda oblast (Kazakhstan), in the middle desert subzone (Kyzylkum) on takyr-like desert solonchak soils.

Study design

At the site of the Dnepr LV accident and adjacent areas, soil samples were collected for genotoxicity assessment using the Allium test system and human lymphocyte culture. In the same area, reptiles and rodents were captured, morphometrically analyzed and peripheral blood samples were taken for cytogenetic analysis. Further, statistical analysis of the obtained data was performed.

Objects

Expeditionary work to the study areas was conducted during the spring and summer of 2022–2023.

A map of the study region and associated areas is presented in Fig. 3.

The Dnepr LV accident occurred in the Karmaksha district of Kyzylorda oblast in the medium desert subzone on takyric desert soils (Veselova et al., 2022), which are defined as “Salic Cambisols (Takyric)” in the World Reference Base for Soil Resources (WRB) system (Lednev et al., 2020b). In the comparison area (Komekbayev settlement area) and background area, a similar type of soil was investigated. The object of the study was 14 pooled soil samples collected on the territory and in the area of the emergency crash site of the Dnepr LV. The chart of characterization of sampling sites is presented in Fig. 4 (Table 1). A system of concentric circles located at an equal distance from the crater center (N 44.342420, E 62.581490) was used for soil sampling. Point samples were collected in April 2023 in 3 repetitions from a depth of 10 ± 5 cm, weighing 250 g. A pooled sample was made by mixing point samples collected from the same site. Samples were bagged, numbered, and transported to the laboratory for testing.

The comparison area for this region is a site near Komekbaev village, without anthropogenic influence (including for preliminary assessment of potential risk to the population), located 40 km from the Big Crater (N 44.825827, E 63.203746). The nature protection area without anthropogenic impact—Altyn Emel Nature National Park—was used as a background area (comparison area) for sampling (sampling location—N 43.940919, E 78.944439).

The most common species of reptiles (species of the genus Eremias) and rodents inhabiting the study area were used as indicator animals. Lizards,

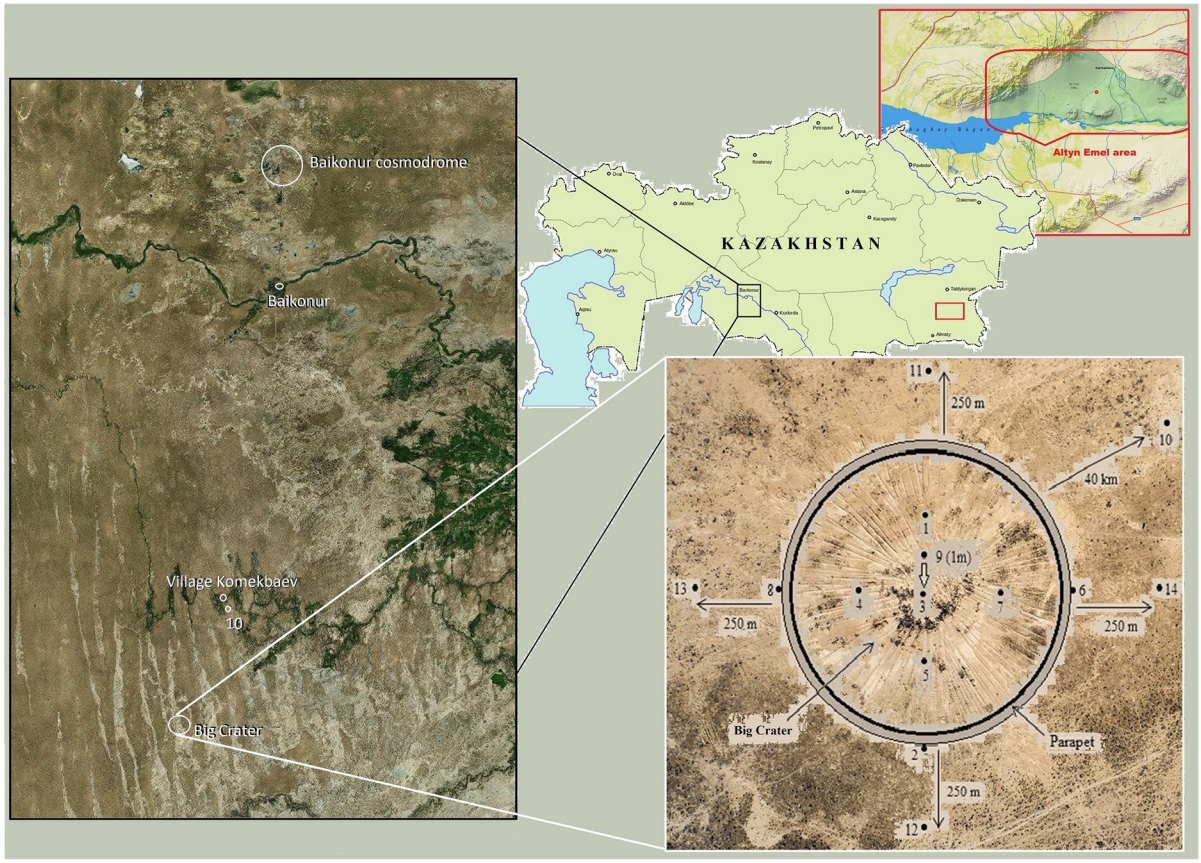

Fig. 3 Map of the study region, associated areas, and scheme of soil sampling in the area of the Dnepr LV crash site based on the satellite image of the Big Crater of the Dnepr LV crash site

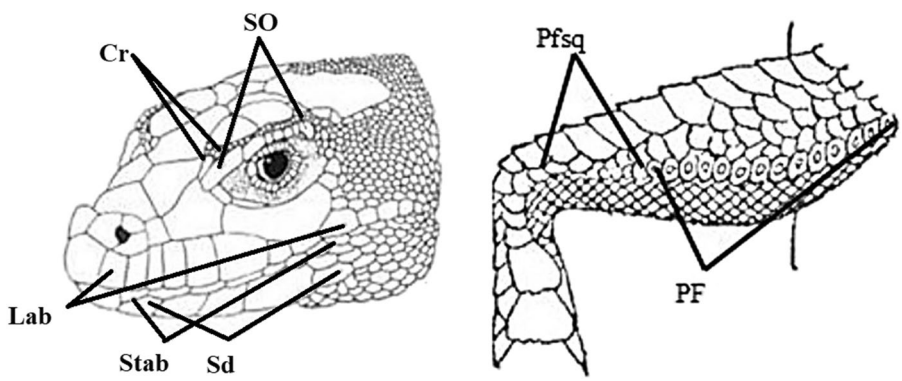

Fig. 4 Schematic of pholidosis of the head and limbs of lizards of the genus *Eremias*

Eremias intermedia, 5 individuals; *Eremias velox*, 5 individuals *Eremias lineolata*, 13 individuals, were captured. Mammals were represented by Great gerbil (*Rhombomys opimus*), 11 individuals; red-tailed gerbil (*Meriones libycus*), 8 individuals; Tamarisk gerbil (*Meriones tamariscinus*), 14 individuals; House Mouse (*Mus musculus*), 7 individuals; Great Jerboa (*Allactaga major*), 9 individuals. Eremias velox, 9 individuals, were captured on the territory near Komekbaeva village. For comparative analysis, we used data of the same species of animals

Table 1 Characteristics of soil sampling locations at the site of the Dnepr LV crash site

№ of samples	Collection site
3	Center
9	Center at a depth of 1 m
1, 4, 5, 7	20 m from the center to the north, west, south, and east
2, 6, 8	Parapet north, east, west
11, 12, 13, 14	250 m from the center of the crater
10	Comparison area—40 km from the crater (the sample was taken near the nearest residential village (Komekbaev), in a place not subject to outside anthropogenic influence)

captured in Aktobe region, Eastern Kyzylkum, Estuary of river Syrdarya, Almaty region.

Preparation of aqueous soil extracts

To analyze the mutagenic activity of soil samples, aqueous soil extracts were prepared (Mouchet et al., 2006; Marcato-Romain et al., 2009). Test soil portions of 30 g each (measuring error < 0.1 g), were placed in glass containers and 150 ml of distilled water was added. The soil and water were mixed for 3 min on a magnetic stirrer and left to settle. The upper fraction was collected and centrifuged for 5 min at 1000 rpm to sediment solid particles (GOST 14.4.4.0284, GOST-29269–91).

The prepared aqueous soil extracts for testing on human lymphocyte cultures were sterilized, but not for the Allium test. Soil taken at a distance of 40 km from the area of the accidental fall of the Dnepr LV (sample № 10) was used as an internal control. Soil taken from a conservation area without anthropogenic impact (comparison area) was used as an external control (Natural National Park "Altyn Emel," Almaty region).

Allium test

Allium test, as a standardized and sensitive test method, is a suitable model to study the environmental and genotoxic potential of pollutants. It has been adopted by the International Plant Bioassay Program (IPPB) for monitoring or testing environmental pollutants and has been widely used for more than forty years to assess water, soil, environmental pollution for various types of pollutants (Leme and Marin-Morales, 2009; Ma, et al., 2005).

To perform the Allium test (AT), *Allium cepa* seeds were germinated in Petri dishes with the test sample at a temperature of 22–24 °C (for 48–72 h). When the roots reached 2–3 cm, the tip 1–2 cm long was cut off. It was placed in a fixative (ethanol/glacial acetic acid—3/1) for 4–24 h. Hydrolyzed in 1N HCl at 60 °C for 8–10 min. Washed in distilled water and stained with orcein solution in a boiling water bath for 6–12 min. Next, crushed samples were prepared according to standard methods, and microscopic analysis ad evaluation of selected cytogenetic parameters were performed (Bianchi et al., 2016; Leme and Marin-Morales, 2009; Rank and Nielsen, 1994). Experiments were performed in three repetitions. At least 3000 cells were examined for each analysis point. Cytogenetic analysis was carried out under a microscope and all occurring abnormalities at different stages of cell division were taken into account. The frequency of micronuclei (Mn,%) was taken into account in interphase cells and assessed to the total amount of analyzed interphases in the sample. At the metaphase stage, micronuclei, dropped chromosomes, and C-metaphases were taken into account. In the anaphase and telophase analysis, the frequency of micronuclei, lagging chromosomes, and bridges at the stages of anaphase and telophase were taken into account to the total sum of anaphase and telophase in the sample. In all variants, the mitotic index was also taken into account.

Cultivation of human lymphocytes and making of samples

To test samples for genotoxicity on human lymphocyte cultures (HLC), blood samples were taken from four healthy volunteer donors from the cubital vein into plastic tubes with lithium heparin. Cell

cultivation and making of samples were carried out according to the following procedure: 0.5 ml of peripheral blood was added to 4.5 ml of a culture medium consisting of 80% RPMI-1640 medium with glutamine (2 mM), 20% cattle serum, penicillin 100 units/ml, and streptomycin 100 units/ml. Lymphocyte division was stimulated with 2% PHA. To analyze the mutagenic activity of the studied samples, 10% of each sample (of the amount of cultivation medium) was added to human lymphocyte cultures 1 h after the start of cultivation for the entire incubation period. Cells were incubated at 37 °C for 48 h in a CO2 incubator.

To accumulate metaphase plates, colchicine was introduced into the culture medium at a final concentration of 0.8 μg/ml 2 h before fixation. To obtain cytological samples, cells were hypotonicized with 0.075 M KCl at 37 °C for 15 min, fixed with a mixture of methyl alcohol/glacial acetic acid (3/1), dropped onto cooled glass slides, and stained with a 4% solution of Giemsa dye. Cytogenetic analysis of human chromosomes was carried out on encrypted samples using a "blind control" method using a motorized light microscope "Zeiss AxioImiger Z.2" with Metafer-4 software. The number of cells with aberrations was determined, as well as it has been analyzed the number and type of aberrations per 100 metaphases (Moorhead et al., 1960).

Experiments were performed in three repetitions. At least 250 metaphases were examined for each analysis point. The spontaneous frequency of chromosomal abnormalities in lymphocytes of healthy donors used for testing the studied samples served as a control.

Faunal studies

The collection of ecological and faunal materials was carried out using methods of observation, capture, and diagnostics accepted in zoology (Poletta et al., 2017; Poletta and Siroski, 2019; McCleery, 2022). Determination of species diversity and identification of an indicator group of animals was conducted on the results of determining profile zoological characteristics and traits in field and laboratory conditions.

Among all the reptile species living in the study area, the most suitable objects for estimation of population development homeostasis are species of *Eremias* genus which is the most abundant and widespread. The collection of materials held in the area of the "Big Crater" and the environs of the village of Komekbaev (adjacent territories). For reference, we used collection material from the Institute of Zoology of the Republic of Kazakhstan (IZ RK, Almaty), and the Zoological Museum of Moscow State University (ZM MSU, Moscow).

To determine the fluctuating asymmetry of bilateral characters in individual species of the genus *Eremias*, 8 characters of pholidosis of the trunk and limbs were studied. The number of upper labial scutes (Lab), the number of lower labial scutes (Slab), the number of superciliary granules (SO), the number of mandibular scutes (Sd), the number of granules in front of the supraorbital scutes (Gr), the number of femoral pores (PF), and the number of scales from the femoral pore (Pfsq) to the bend of the knee (Fig. 4). Small elements of pholidosis were recorded using a binocular loupe. Digital calculations were implemented in the Statistica-10 program. An analysis of external morphological characteristics for anomalies was also carried out.

Among mammals, rodents are a generally recognized indicator group of animals. Important elements characterizing the state of indicator species of rodents are the ecological features of habitat and stay, as well as a set of indicators of habitus and functional state.

Determination of a complex of such exterior and interior indicators in widespread animal species serves as one of the objective forms of indication of the condition of mammals and living conditions in the area of the Dnepr launch vehicle accident. To study their morphological and functional parameters, rodents were caught using traditional schemes for using live traps on small mammals.

To assess the condition of animals in this group, morph-functional (exterior and interior) characteristics were determined (Dallas et al., 2020), which represent a set of indicators of individuals of each indicator species captured in the summer season and living in places relatively distant from each other. Determination of exterior characteristics was carried out according to the scheme of metric indicators of animals: p, weight (g); l, body length; c, tail; a, ear height; and pl, foot length (in mm). Exterior indicators characterize the specific characteristics of the animal's habit in certain living conditions.

Functional characteristics (interior) were determined by the indices of internal organs (ratio of organ

mass to body weight, ‰): il, liver; ih, heart; ik, kidneys; and is, spleen. These indicators reflect the characteristics of the physiological state for each animal species, associated mainly with the level of basal metabolism, the specifics of nutrition, age, season, and geographical habitat.

Recording environmental features, exterior and interior characteristics makes it possible to establish species indicators, both corresponding to natural systematic characteristics and functional capabilities, and to determine deviations from normal indicators that are characteristic of animals living in Kazakhstan. The presence of high negative environmental loads manifests itself in animals in the form of significant deviations in morph-functional indicators.

Cytogenetic studies of animals

To identify the mutagenic effect on living organisms as a result of exposure to an unfavorable environmental situation, cytogenetic characteristics of the blood of the most common lizard representatives in the study region were used as biomarkers.

The object of the study was natural populations of lizards of the genus Eremias (8 animals) and mammals—rodents (field mice, voles, sandflies) (10 animals), and gerbils (4 animals), which were captured in the area of the Dnepr LV accident. To assess the spread of the impact of the consequences of the Dnepr LV accident, animals were also captured 40 km away from its epicenter (lizards, 4 animals; rodents, 5 animals). For cytogenetic studies, we used data from animals captured in the background area without anthropogenic influence, with similar natural and climatic conditions (Almaty region) (lizards, 6 individuals; rodents, 14 individuals). Three to four blood smears were prepared from each animal.

To perform laboratory hematological studies (preparation and analysis of cytogenetic smears), biological samples (peripheral blood) were taken. Blood collection and smear preparation were carried out using the generally accepted method in the field, without removing animals from the environment (Poletta and Siroski, 2019; Schmid, 1975; Mitkovska et al., 2012).

Micronucleus analysis in rodents can be performed in cells of any proliferating tissue, but it is easiest to detect them in erythrocytes (blood cells without a basic nucleus), especially since blood is the most accessible tissue for examination and usually does not require killing the animal. Blood erythrocytes in a sample are classified into polychromatophilic (immature) or normochromic (mature). In acute exposure assessment, it is preferable to assess micronucleus frequency in polychromatophilic erythrocytes, and in environmental monitoring or chronic exposure studies in the total peripheral blood erythrocyte population. The average lifespan of erythrocytes is 3–4 months, which allows the most complete assessment of the impact of the factors under study. Since mammalian peripheral blood erythrocytes are nuclear-free, the main type of cytogenetic disorder is micronuclei, which are seen in the microscope as rounded or oval things of different sizes densely stained with a clear contour. Since erythrocytes represent the last stage of erythropoiesis, being the final product of several blast cell divisions, after the last mitosis the main nucleus is pushed out, while micronuclei resulting from chromosomal abnormalities in erythroblast cells remain in the cytoplasm.

Cameral processing of the samples was carried out in laboratory conditions. Peripheral blood smears were fixed in 96% ethyl alcohol for 30 min, dried, and stained with 4% Romanovsky-Giemsa solution for 20 min.

The frequency of micronuclei and cytological disorders were taken into account in normochromic erythrocytes of peripheral blood on a Zeiss AxioLab A.1 microscope under oil immersion and magnification 10×100. During a cytogenetic examination, all disturbances in the structure of erythrocytes that differed from the normal morphology characteristic of this species were recorded. Up to 10,000 red blood cells were examined from each individual studied. Photo documentation was carried out of the most characteristic disorders of peripheral blood erythrocytes.

Statistical analysis

For statistical analysis, the arithmetic mean and its deviation ($M \pm SE\%$) were calculated. The reliability of the mean difference was assessed on the basis of the Student's t-criterion and Mann–Whitney U test. A statistical significance threshold of $p \leq 0.01$ was applied. Statistical and correlation analysis of data was performed using Excel (Microsoft Corporation,

Redmond, Washington, DC, USA). Data were tested using Pearson correlation and Spearman correlation.

Results

The observations have shown that despite the relatively long period of time since the accident, there has been no recovery of the natural soil, and vegetation cover in the area is in the early stages of recovery (Atygayev et al., 2021; Veselova et al., 2022). This is also confirmed by the presence of relief transformation in the form of wind and water erosion, as well as by the preserved traces of crater planning and road degradation (Fig. 2). Elements of degradation in the basic segment of environmental objects (soil, vegetation cover) clearly indicate the remote consequences of the Dnepr LV accident.

Study of soil genotoxicity

The data of the genotoxicity study of aqueous extracts of soils sampled at the site of the Dnepr LV crash and the adjacent territory by Allium test methods and on human lymphocyte cultures are presented in Tables 2 and 3.

Cytogenetic analysis of samples in human lymphocyte cultures (HLC) revealed aberrations of chromosomal and chromatid types with a predominance of chromatid-type disorders in the form of single breaks characteristic of chemical factors (Fig. 5).

In Allium test (AT), all occurring cytogenetic disorders at different stages of cell division were taken into account. In interphase cells, micronuclei were the main type of abnormalities. At other stages of mitosis (metaphase, anaphase, telophase), micronuclei and chromosomal abnormalities including

Table 2 Genotoxicity data of soil samples on human lymphocyte culture

№ of samples	Cells analyzed	Cells with aberrations, %	Total aberrations, %	Chromosomal type, %	Chromatid type, %
Inside the crater					
3	635	2.05 ± 0.40	2.05 ± 0.40	0.79 ± 0.25	1.26 ± 0.31
1	272	1.47 ± 0.36	1.47 ± 0.36	0.37 ± 0.18	1.1 ± 0.32
4	417	1.44 ± 0.41	1.44 ± 0.41	0.24 ± 0.17	1.2 ± 0.38
5	603	1.49 ± 0.35	1.82 ± 0.38	0.83 ± 0.26	0.99 ± 0.28
7	294	0.68 ± 0.24	0.68 ± 0.24	0	0.68 ± 0.24
M ± SE	2221	1.43 ± 0.25	1.49 ± 0.25*	0.45 ± 0.14	1.04 ± 0.21
Center at a depth of 1 m					
9	430	3.02 ± 0.58	3.95 ± 0.66*	2.56 ± 0.54	1.39 ± 0.40
Parapet					
2	251	2.79 ± 0.52	3.19 ± 0.55	0.40 ± 0.20	2.79 ± 0.52
6	500	0.6 ± 0.24	0.60 ± 0.24	0	0.60 ± 0.24
8	630	1.27 ± 0.31	1.43 ± 0.33	0.16 ± 0.11	1.27 ± 0.31
M ± SE	1381	1.55 ± 0.33	1.74 ± 0.35*	0.19 ± 0.12	1.55 ± 0.33
250 m from the center of the crater					
11	326	1.53 ± 0.40	1.53 ± 0.40	0.61 ± 0.25	0.92 ± 0.31
12	450	1.33 ± 0.38	1.55 ± 0.41	0.44 ± 0.22	1.11 ± 0.35
13	248	0.81 ± 0.28	0.81 ± 0.28	0	0.81 ± 0.28
14	320	0.94 ± 0.31	0.94 ± 0.31	0.31 ± 0.18	0.63 ± 0.25
M ± SE	1344	1.15 ± 0.29	1.21 ± 0.30	0.34 ± 0.16	0.87 ± 0.25
10 (comparison area—40 km from the crater	380	0.53 ± 0.21	0.53 ± 0.21	0	0.53 ± 0.21
Background area	600	0.57 ± 0.31	0.57 ± 0.31		0.57 ± 0.31
Spontaneous level	600	0.50 ± 0.20	0.50 ± 0.20	0	0.50 ± 0.20

*$p \leq 0.01$ between the investigated indicators and comparison area

Table 3 Data of genotoxicity determination of soil aqueous extract samples using Allium test

№ of samples	Cells analyzed	MN in interphase, %	Metaphase disorders, %	Ana-telophase disorders,%	MI
Inside the crater					
3	3075	2.35 ± 0.27	5.88 ± 2.36	4.17 ± 2.0	6.74
1	3033	1.30 ± 0.21	7.40 ± 2.5	2.00 ± 1.40	5.68
4	3066	1.63 ± 0.23	4.5 ± 2.08	3.33 ± 1.78	8.9
5	3009	1.68 ± 0.23	5.26 ± 2.24	5.5 ± 2.28	7.68
7	3018	0.64 ± 0.14	0	0	7.36
M ± SE	15,201	1.52 ± 0.1	4.61 ± 0.17	3.00 ± 0.14	7.27
Center at a depth of 1 m					
9	3195	7.05 ± 0.45*	23.07 ± 4.02	30.00 ± 4.58	4.04
Parapet					
2	3096	3.01 ± 0.31	10.54 ± 3.1	8.00 ± 2.71	8.43
6	3033	1.07 ± 0.19	6.67 ± 3.65	3.4 ± 1.79	7.12
8	3042	1.66 ± 0.23	5.0 ± 1.99	5.00 ± 2.18	5.16
M ± SE	9171	1.91 ± 0.14*	7.40 ± 0.27	5.47 ± 0.24	6.9
250 m from the center of the crater					
11	3030	1.23 ± 0.20	5.26 ± 2.24	2.63 ± 1.60	10.69
12	3039	1.86 ± 0.24	4.54 ± 2.08	4.00 ± 1.96	8.59
13	3030	0.87 ± 0.17	2.94 ± 1.94	4.54 ± 2.08	8.71
14	3018	0.80 ± 0.16	5.13 ± 2.01	2.63 ± 1.60	12.13
M ± SE	12,117	1.19 ± 0.10*	4.47 ± 0.19	3.45 ± 0.16	10.03
10 (comparison area—40 km from the crater	3009	0.21 ± 0.08	0	0	15.33
Background area	3000	0.15 ± 0.06	1.43 ± 0.22	0.67 ± 0.3	19.00
Spontaneous level	3000	0.10	1.33 ± 0.21	0	25.00

*$p \leq 0.01$ between the investigated indicators and comparison area

MN micronuclei

fragments resulting from deletions and translocations, bridges, lagging, or dropped chromosomes associated with division spindle damage or disordered chromosome behavior on the division spindle were encountered (Fig. 6).

The average frequency of micronuclei and chromosomal aberrations induced by samples taken inside the crater, including the center (samples № 1, 3, 4, 5, 7) is 1.52 ± 0.10% (AT) and 1.49 ± 0. 25% (HLC), on the edges of the crater (former barnacle) (samples № 2, 6, 8)—1.91 ± 0.14% (AT) and 1.74 ± 0.35% (HLC). The average frequency of violations in the study of samples taken at a.

distance of 250 m from the crater (samples № 11, 12, 13, 14) is 1.19 ± 0.10% (AT) and 1.21 ± 0.30% (HLC). The highest frequency of detected violations was recorded in the study of soil samples taken in the center of the crater at a depth of 1 m (sample № 9) 7.05 ± 0.45% (AT) and 3.95 ± 0.66% (HLC). The lowest genotoxicity was observed in the study of the sample taken 40 km from the place of the launch vehicle impact (sample № 10) 0.21 ± 0.08% (AT) and 0.53 ± 0.21% (HLC) (internal control). Assessment of statistical significance showed reliable differences in the values of the studied samples taken inside the crater, its edge and at a distance of 250 m compared to the data of internal and external control ($p \leq 0.01$).

A decrease in mitotic index was observed in all samples compared to the control. A negative correlation (−0.57, $p \geq 0.05$) between the frequency of micronuclei in the interphase and the value of mitotic index in Allium test was revealed, in other words, the higher the level of genotoxicity of samples, the lower the ability of cells to divide in the apical meristem.

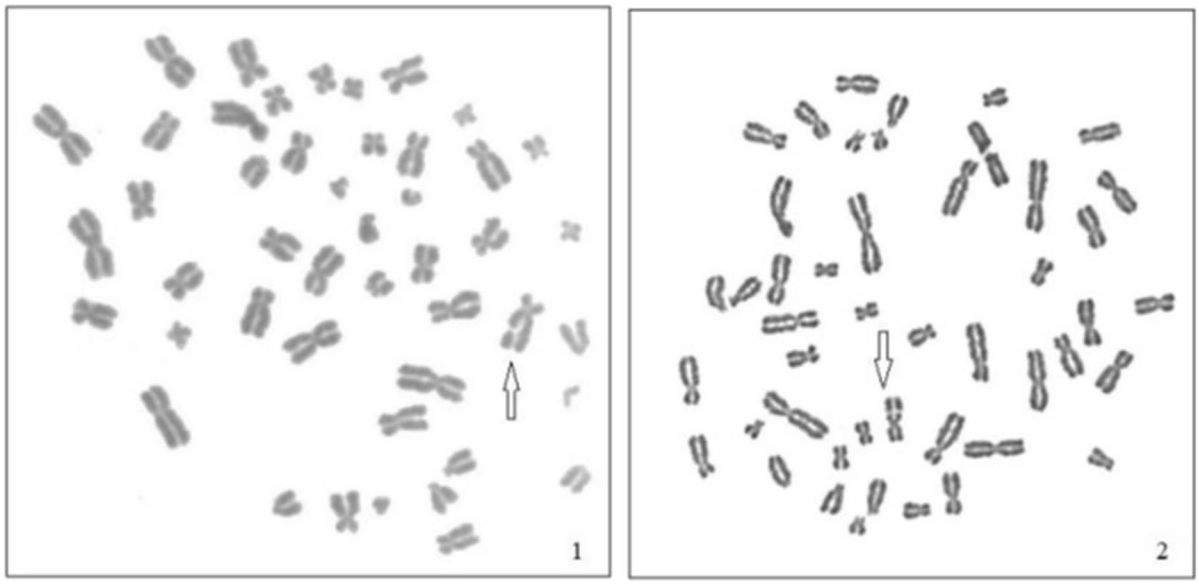

Fig. 5 Types of chromosomal disorders detected during cytogenetic analysis of soil samples in human lymphocyte cultures (HLC). 1, chromatid break; 2, chromosomal disruption

The in vitro tests used showed a similar rank degree of contamination of the studied samples, despite their different sensitivity. Therefore, a correlation analysis of the results of cytogenetic analysis of testing the studied samples on human lymphocyte cultures and the Allium test was carried out. It showed a statistically significant relationship between the frequency of aberrations in lymphocytes and micronuclei in interphase cells—0.92, the frequency of chromosomal aberrations and disorders in metaphase—0.88, and the frequency of chromosomal aberrations and disorders in ana-telophase analysis—0.83, $p \leq 0.01$ (Fig. 7).

Species diversity of herpetofauna in the studied territories

According to literature, archival data and collection materials, the reptile fauna in the emergency area and its geographically close surroundings (Northern Kyzylkum) is represented by 21 species (Leme and Marin-Morales, 2009; Fenech et al., 2011; Guilherme et al., 2008; Poletta et al., 2013).

During the period of collection at the sites during the spring–summer survey season, the presence of 1 amphibian and 8 reptile species was noted:

1. Perrin's toad (*Bufotes perrini*).
2. Central Asian turtle (*Testudo horsfieldii*).
3. Squeaky gecko (*Alsophylax pipiens*).
4. Steppe agama (*Trapelus sanguinolentus*).
5. Takyr roundhead (*Phrynocephalus helioscopus*).
6. Rapid lizard (*Eremias velox*).
7. Medium-sized lizard (*Eremias intermedia*).
8. Lineated lizard (*Eremias lineolata*).
9. Arrow snake (*Psammophis lineolatus*).

Three species of the genus *Eremias* were the most abundant and widespread in the Dnepr LV accident sites. The established indicators of systematic diversity and occurrence of herpetofauna representatives were close to those known previously for this and geographically close areas (Poletta et al., 2017; Poletta and Siroski, 2019; Leung et al., 2022; Zakharov, 2001; Pollo, 2015).

Morphophenetic study of reptiles

The species selected as indicator species (*Eremias intermedia*, *Eremias lineolata*, *Eremias velox*) were analyzed for variability of morphological characters, as well as for fluctuating asymmetry. Comparison of folidosis variation showed no fundamental differences from other compared samples.

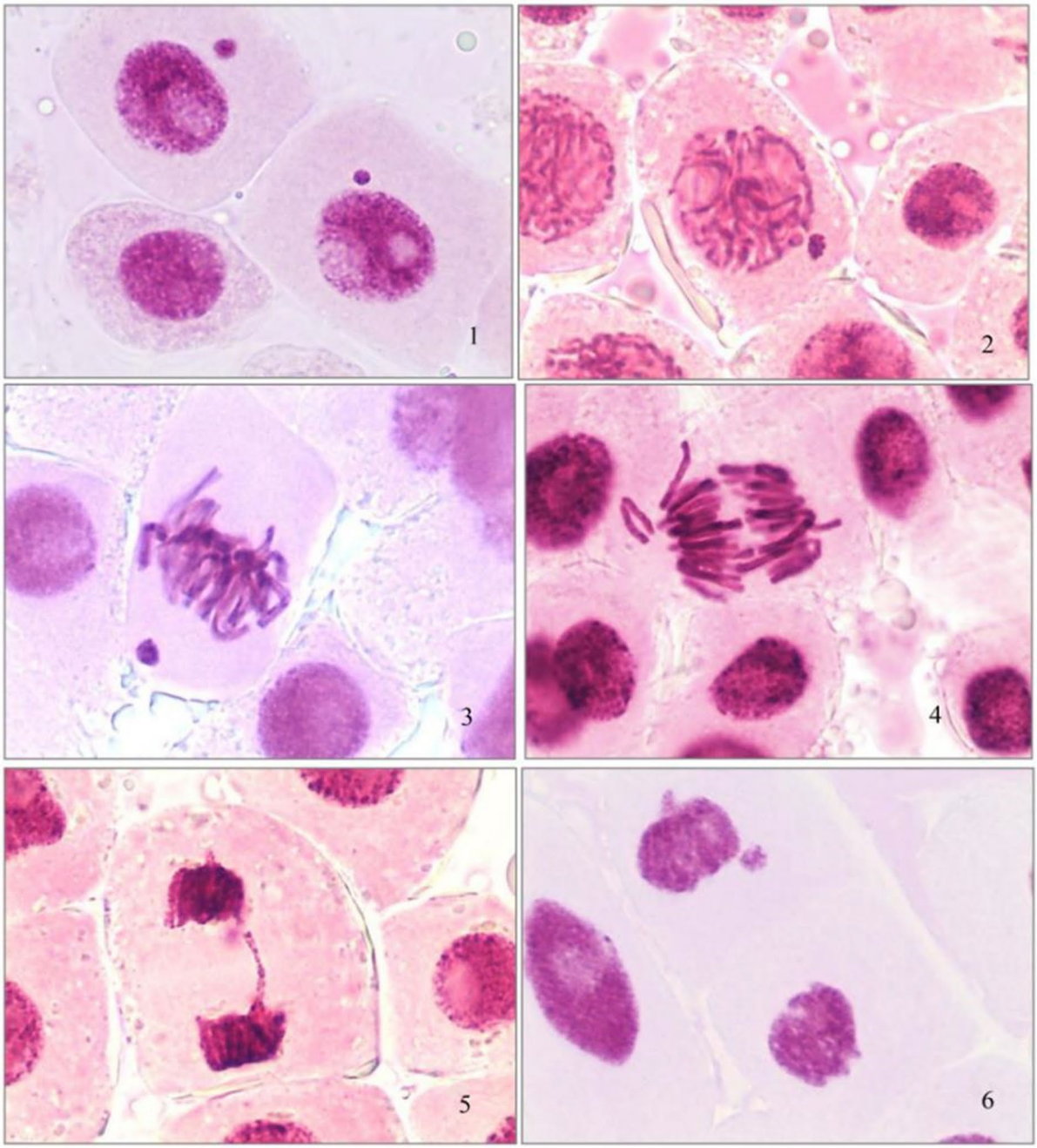

Fig. 6 Types of cytogenetic disorders in the Allium test. 1, interphase cells with micronuclei; 2, micronucleus in prophase; 3, micronucleus in metaphase; 4, dropped out chromosome in anaphase; 5, chromosome bridge in telophase; 6, micronucleus in telophase

A number of publications from the practice of monitoring studies have shown that reptiles of the family Lacertidae are one of the convenient objects of bioindication. The example of the leaping lizard—Lacerta agilis clearly demonstrates the use of the assessment of the fluctuating asymmetry of reptiles for ecological monitoring (Leung et al., 2022; Zakharov, 2001; Blum and Ott, 2018). There are also works devoted to the study of the fluctuating asymmetry of other representatives of the family—the

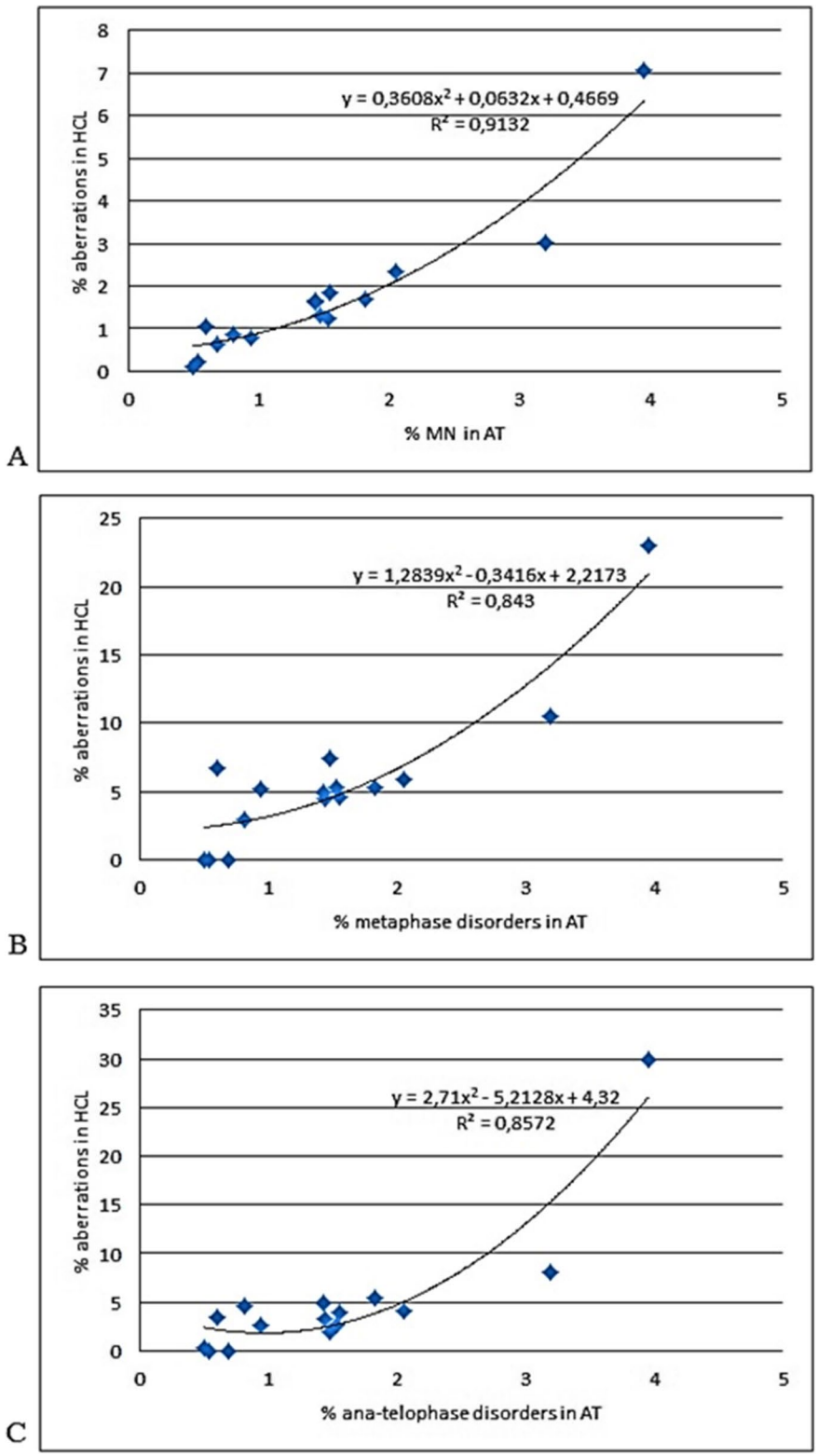

Fig. 7 Scatter diagram showing correlation and polynomial dependencies between the data on genotoxicity assessment of soils sampled in the Dnepr LV accident area using HLC and AT test systems. **A** frequency of chromosomal aberrations in HLC and micronuclei in interphase cells in AT; **B** frequency of chromosomal aberrations in HLC and disturbances in metaphase in AT; **C** frequency of chromosomal aberrations in HLC and disturbances in ana-telophase in AT

variegated lizard Eremias arguta (Chirikova, 2014) and the swift lizard (Chirikova and Khamitov, 2015).

The values of the frequency of fluctuating asymmetry on the trait, as one of the indicators characterizing the degree of well-being of the existence of populations, are shown in Table 4.

It should be noted that there is still no scale for the assessment of such an indicator as asymmetry per trait and, in general, the condition of *Eremias* can only be assessed by comparison. The comparative data in Table 4 show that the two species of the genus *Eremias* inhabiting the survey area had a slightly higher level of mean fluctuating asymmetry. Thus, for the lineated lizard, the asymmetry per trait was 0.312, and for the medium lizard, it was 0.33.

Ecological and morphofunctional indicators of rodents

A group of indicator rodent species was formed based on the results of trapping. In the catches, the comb gerbil (*Meriones tamariscinus*), great gerbil (*Rhombomys opimus*), and red-tailed gerbil (*Meriones libycus*) dominated more often, while the greater jerboa (*Allactaga major*) and house mouse (*Mus musculus*) were caught less frequently. This composition of rodents and frequency of occurrence in catches at the "Dnepr" rocket accident site corresponds to the regional habitat of these species. It was shown that in the case of mass trapping, the species composition of the rodents was as follows in terms of representativeness among rodents: greater gerbil, comb gerbil, red-tailed gerbil, yellow gopher, and house mouse.

Observations of the behavior and habitat conditions of indicator rodent species were carried out at the survey sites. It was shown that the daytime activity of rodents in the surveyed areas was normal and was observed mainly at night, in the evening, and morning twilight. The nature of feeding was without any distinctive features and was determined mainly by climatic conditions and food availability. The diet of captured animals was mainly formed of green fodder. Gerbil colonies and burrows had a regular architecture and shape of arrangement according to the specific terrain and soil characteristics. Observed and captured indicator animals had, as a rule, typical coloration for each of the species inhabiting different regions of Kazakhstan. The only exception was comb gerbils, which had a brighter and more contrasting coloration than animals inhabiting other regions of the country, which corresponds to the data given in the summary "Mammals of Kazakhstan."

Thus, the survey of the Dnepr LV accident site showed that there are no fundamental differences in the nature of stay, feeding, behavior, activity, and settlement sites between the captured animals and animals of these indicator species from other regions of Kazakhstan.

A set of metric and functional characteristics was determined in captured rodents to determine the main exterior and functional parameters. The results of the laboratory study provided data on

Table 4 Data of asymmetric manifestation on a trait of 3 species of the genus *Eremias*

Samples	*n*	Mean	Min	Max	Standard error
Medial racerunner (*Eremias intermedia*)					
"Big Crater"	5	0.33	0.2	0.4	0.066
Aktobe region, station Shalkar	22	0.206	0.000	0.400	0.024
Eastern Kyzylkum	22	0.182	0.000	0.400	0.023
Rapid racerunner (*Eremias velox*)					
"Big Crater"	5	0.25	0.1	0.4	0.064
Village Komekbaeva	8	0.203	0.000	0.400	0.042
Estuary of river Syrdarya	9	0.266	0.100	0.400	0.021
Eastern Kyzylkum	13	0.161	0.000	0.300	0.031
Striped racerunner (*Eremias lineolata*)					
"Big Crater"	13	0.312	0.100	0.500	0.042
Aktobe region, station Shalkar	7	0.213	0.000	0.400	0.035
Eastern Kyzylkum	10	0.220	0.100	0.400	0.038

n number of individuals in the sample, *Mean* arithmetic mean, *Min* minimum value of the feature, *Max* maximum value of the feature, *Std.Err* standard error of the mean

Table 5 Selected morphofunctional indices of indicator species

Species	Number of animals	Metrics	Accident site of Dnepr	Kazakhstan regions*
			Limit	Limit
Great gerbil *Rhombomys opimus*	11	weight (g)	180–192	182–210
		Body length (mm)	171–180	162–187
		Liver index (‰)	40–57	36–68
		Heart index (‰)	3.1–3.5	2.9–3.5
		Kidney index (‰)	4.4–6.1	4.2–6.8
Red tailed gerbil *Meriones libycus*	8	weight (g)	92–128	92–136
		Body length (mm)	135–163	130–170
		Liver index (‰)	49–52	46–57
		Heart index (‰)	4.0–4.2	3.9–4.3
		Kidney index (‰)	5.6–6.4	5.4–6.9
Tamarisk Gerbil *Meriones tamariscinus*	14	weight (g)	120–144	112–147
		Body length (mm)	153–167	150–169
		Liver index (‰)	41–52	38–55
		Heart index (‰)	3.8–4.1	3.8–4.4
		Kidney index (‰)	5.4–5.9	5.0–5.9
House mouse *Mus musculus*	7	weight (g)	12.3–16.3	11–19
		Body length (mm)	74–86	62–89
		Liver index (‰)	50–64	40–68
		Heart index (‰)	7.3–8.1	5.9–12.6
		Kidney index (‰)	8.8–9.2	6.1–10.1
Great Jerboa *Allactaga major*	9	weight (g)	237–378	233–470
		Body length (mm)	179–202	174–220

*From data summary "Mammals of Kazakhstan" (Mitkovska et al., 2012; Blum and Ott, 2018; Chirikova, 2014)

interior and exterior parameters. The following indices were determined in indicator species inhabiting the area of Big Crater and Komekbaev settlement: weight, body length, and internal organ indices (Table 5). Comparative analysis was carried out with similar indicators for species inhabiting different regions of Kazakhstan. Data are given as limits of variation of values registered for animals of each species.

Exterior characteristics of captured animals were without peculiarities and corresponded to the limits of variation of indicators of animals inhabiting Kazakhstan and its southern region, in particular. The metric parameters and their ratios were not distinguished from those generally accepted for the species of these animals and corresponded to sex and age norms. The exterior of the main group of indicator species of rodents meets the main ecological and systematic criteria of these animals.

Animals of indicator species captured in the area of Big Crater and Komekbaev settlement had no pathological features. Only one red-tailed gerbil captured near the Big Crater had an echinococcus-like mass in the liver.

Values and variability of indices of internal organs of voles, as indicators of their physiological state, reflect mainly the level of basic metabolism. The limits of values of a number of indices (liver, heart, kidney indices) of rodents captured in the area of the Dnepr LV accident generally lie within the limits of variation for these species in Kazakhstan.

Thus, the study of individuals of 4 species of rodents showed that the variability of the determined values of the main metric and functional indicators of the captured animals do not show any deviations from the norm and generally correspond to similar characteristics of these species of animals living in Kazakhstan (Panteleev et al., 1990).

Table 6 Results of cytogenetic analysis of the genus *Eremias* erythrocytes

Variant	Number of animals	Cells analyzed	MN, %	Nuclear invaginations, %	Amitosis, %	Tail, %	Vacuole, %
Accident area of Dnepr LV	8	80,000	0.27 ± 0.0185*	0.17 ± 0.015*	0.05 ± 0.008*	0.20 ± 0.016*	0.09 ± 0.01
Comparison area—40 km from accident of Dnepr LV	4	40,000	0.15 ± 0.05	0.12 ± 0.024	0.03 ± 0.01	0.05 ± 0.01	0
Background area	6	60,000	0.023 ± 0.011	0.09 ± 0.017	0.02 ± 0.009	0.01 ± 0.005	0

*$p \leq 0.01$ between the investigated indicators and background area data

MN micronuclei

Studyof the genetic status of FMD and rodents using the micronucleus test

Cytogenetic indices characterizing the genetic status of representatives of herpetofauna were determined for three indicator species from the genus *Eremias*.

The results of the performed analysis in blood erythrocytes of the examined animals are presented in Table 6.

According to the literature data in control populations of bipedal (*Darevskia raddei*) and parthenogenetic lizards (*Darevskia armeniaca*) the level of micronuclei in erythrocytes is in the range of 0.06 ± 0.04–0.20 ± 0.13% (Simonyan et al., 2018). The frequency of micronuclei in erythrocytes of the control population of the genus *Eremias* was 0.023 ± 0.011%, which is below the specified limits, considering possible species differences. The frequency of micronuclei in *Eremias* caught in the Dnepr RN accident area was statistically significantly higher than the control level (more than tenfold) ($p \leq 0.01$). Animals caught 40 km from the accident site also showed an increased level of cytogenetic disorders. This corresponds to the detection of NDMA (heptyl transformation product) in plants near Komekbayev (Bisarieva et al., 2012) and the deterioration of public health in the territories adjacent to RN accident areas (Kozlovsky et al., 2020).

When performing micronuclei analysis in its extended form, the so-called cytomic analysis in any type of nuclear cells, in addition to micronuclei there is also a rather wide range of morphologic types of nuclear abnormalities (Krivtsova et al., 2021). These include nuclear budding (invagination of the nuclear envelope), formation of nucleoplasmic bridges, "tailed nuclei," protrusions of various types, amitosis, etc. Nuclear anomalies indicate not only degenerative processes in the cells in which they are observed, but also the fact of previous chromosomal aberrations, as a result of which they were formed.

The greatest percentage of disturbances in the structure of erythrocytes of peripheral blood, in the examined lizards is associated with cytological disturbances. They, as well as micronuclei, significantly exceed the control level.

The main cytological disorders were invagination (budding and invagination) of the nuclear envelope, amitosis, and "tail" erythrocytes. There were erythrocytes with vacuoles, which were not recorded in the control group. Binuclear erythrocytes, erythrocytes containing two nuclei connected by one or more pulls (bridges); and nuclear-free erythrocytes, macro- and microcytes were also encountered (Fig. 8).

The results of the cytogenetic analysis of erythrocytes of rodents in the area of the Dnepr LV crash are presented in Table 7.

The frequency of micronuclei in erythrocytes of peripheral blood of rodents caught in the area of the LV accident statistically significantly ($p \leq 0.01$) differs from the results obtained when analyzing rodents caught in control regions (0.05%) and twice exceeds the similar indicators of animals caught 40 km from the accident site (Komekbaev village). In about 30% of cases, micronuclei were quite large, and erythrocytes with two micronuclei were also recorded. Some studies have shown that approximately 0.025 ± 0.011% of micronuclei are detected in erythrocytes of white mice (Zakharov, 2001; Pollo, 2015),

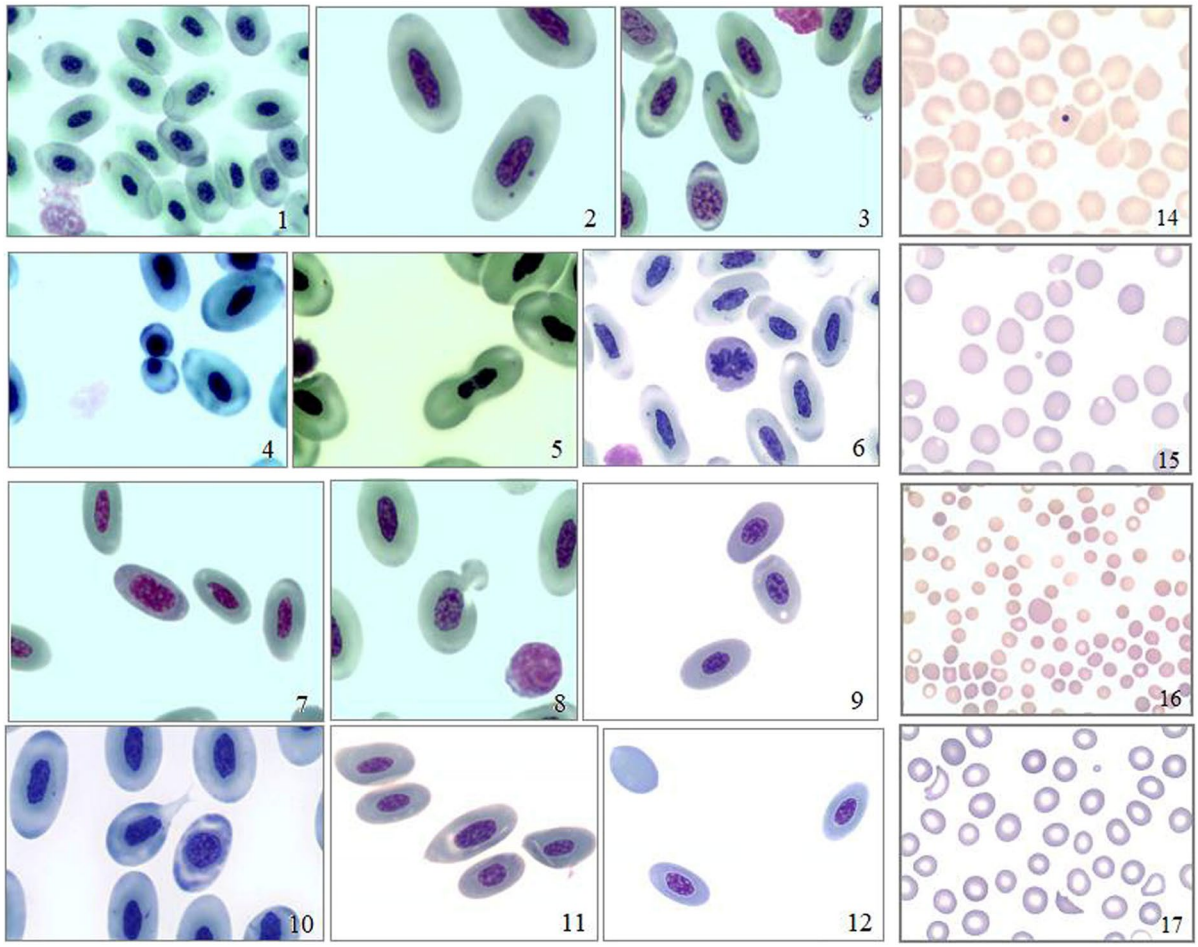

Fig. 8 Cytogenetic and cytological disorders in erythrocytes of lizards and rodents (1, erythrocyte with micronucleus; 2, erythrocyte with two micronuclei; 3, erythrocyte with detached micronuclei; 4 and 5, amitosis; 6, cell in the bloodstream at the stage of mitosis; 7, macrocyte with enlarged nuclear content and invagination of the nucleus; 8, poikilocytosis; 9, vacuolysis; 10, erythrocyte with "tail"; 11, macrocyte; 12, nuclear-free erythrocyte); 13, gerbil erythrocyte with micronucleus; 14, microcyte in peripheral blood of house mouse; 15, macrocyte in peripheral blood of house gerbil; 14, poikilocytosis and microcyte in peripheral blood of house mouse), magnification 16×100

Table 7 Results of cytogenetic analysis of erythrocytes of rodents captured in the area of the Dnepr LV crash site

Trapping locations	Number of animals	Number of checked cells	MN, %	Cytological disorders, %
Mouse-like rodents (accident area of Dnepr LV)	10	100,000	0.28 ± 0.053*	0.28 ± 0.053*
Mouse-like rodents (comparison area—40 km from accident of Dnepr LV)	5	50,000	0.15 ± 0.017*	0.11 ± 0.015*
Mouse-like rodents (background area)	14	140,000	0.05 ± 0.008	0.095 ± 0.012
Jerboas (accident area of Dnepr LV)	4	40,000	0.35 ± 0.029*	0.25 ± 0.025*
Jerboas (background area)	4	40,000	0.15 ± 0.019	0.09 ± 0.015

*$p \leq 0.01$ between the investigated indicators and background area data

MN micronuclei

which corresponds to the data obtained from comparison groups.

The frequency of micronuclei in the erythrocytes of *Allactaga major* captured by the study area was more than two times higher than the control values.

Among cytologic disorders there were fixed erythrocytes of non-standard size, as a rule, these are small (microcytes) or large (macrocytes) erythrocytes and, in separate cases, poikilocytosis—change of normal shape, thickness, and volume of cells (Fig. 8).

The obtained data indicate a significant anthropogenic impact on the genetic apparatus of indicator animals living near the LV accident areas.

Discussion

There were performed studies within the monitoring of environmental objects at the impact site of failed Dnepr LV to assess more fully the long-term effects of the accident. The work involved defining the current state of the crash site, habitat conditions, morphophenetic description, and genetic status of indicator animals (reptiles and mammals). This integrated approach allowed a detailed evaluation of the overall accidental (chemical, physical) and post-accidental impact factors (detoxification and reclamation).

A variety of mutagenic factors are known to interplay with potential targets and such interaction, in turn, features multi-level components (environment, organism, tissue, and cell) and by a number of differently directed effects of the targeted implementation of impact.

Due to the no viability of experimental research on all the possible ways for assessing the potential mutagenicity of complex mixtures and combined mutagenic effects, it is necessary to evaluate the cumulative mutagenicity in the environment. Moreover, the problems associated with the influence of the rocket and space industry (RSI) and launch emergency situations include not only direct toxic effects at the time of the accident, but also its long-term consequences. According to the data obtained at the site of the Dnepr LV accident, there is an increased genotoxicity of soil samples collected in the territory of the crater and at a diameter of 250 m from its edge, especially at a depth of 1 m. In this area, many years after the accident, only the development of local pioneer groups of some weed plants (Fig. 2). Our study of surface soil samples inside and at the edges of the crater l revealed a total excess of mutagenic activity by 3 or more times compared to external and internal controls and ≤ 3 times compared to areas located at a distance of 250 m from the edge of the crater. This is consistent with the data on the scattering of the bulk of the soil from the crash site within a radius of up to 500 m and the detection of the main fragments of the fallen rocket in an area with a radius of approximately 400–500 m.

Sensitivity and correlation studies between test systems used to analyze the potential hazards of genotoxicants are fundamental to more accurately assessing environmental risks. The sensitivity of the test systems used is different and depends not only on its resolution, but also on the cells or tissues being studied (Giri et al., 2002; Şekeroğlu et al., 2013; Abhishek et al., 2014; Feretti et al., 2008). Therefore, there is no single method that allows to unambiguously assess the genotoxic potential of the agent under study, which makes it necessary to use a set of methods performed on different test objects in vitro and in vivo (from microorganisms and higher plants to human and animal cell cultures).

Among the significant values of correlations of data of different test objects used, we should note the correlation between the frequency of chromosomal aberrations in the analysis of soil samples on human lymphocyte cultures and the frequency of micronuclei in interphase cells, disturbances in metaphase and ana-telophase in Allium test (0.83–0.93, $p \leq 0.01$). Similarly, according to the literature, Allium test shows good correlation with tests used on mammals. Thus, Rank and Nielsen (1994) showed a correlation of 82% for carcinogenicity test in rodents and Allium test.

Modern ecology is increasingly focused on natural diagnostic tools using bioindicator species, which are the most common species for this territory. The observations carried out and, in general, the collection and processing of ecological and faunal materials indicate that the species diversity, as well as the features and nature of the stay of animals of the herpetocomplex and mammals (rodents) are in accordance with the indicators of their natural state characteristic of the natural and climatic conditions of the survey period. For an objective environmental assessment of the possible consequences of the accident, an indicator group of three species of *Eremias* lizards and four

species of rodents was identified, in which comparative morphophenetic and fluctuating asymmetry indicators were determined, as a reflection of the degree of well-being of the populations.

As a result, the morphophenetic analysis of *Eremias* lizards showed that there were no statistically significant differences between the control samples and individuals from the emergency territory of the same species, that is, the level of fluctuating asymmetry was similar between the control and samples from the emergency territory. However, a slightly higher level of fluctuating asymmetry was detected in the 2 species of the genus *Eremias* (average and lineated). This may indicate not very stable development of these populations during the periods of material collection (Lajus, 2023; Anđelković et al., 2023; Mirč et al., 2019; Băncilă et al., 2010). This is also common in populations with less genetic variation and therefore higher levels of inbreeding. It is likely that other environmental factors modulate this relationship (Laia et al., 2015).

The comparative analysis of field and ecological traits and morph-functional characteristics of rodents captured at the Dnepr LV accident site in terms of the values of metric indicators and indices of internal organs correspond to their taxonomic and functional features, which are within the limits of variation of traits of specific species inhabiting Kazakhstan.

Thus, the totality of exterior and interior ecological indicators testifies to the satisfactory state of rodent and the genus *Eremias* populations in the area affected by the crash of the Dnepr LV and no significant deviations from the norm at the organismal level have been detected.

Genotoxicity of NDMH, rocket fuel of Dnepr LV and its transformation product NDMA, has been shown in many studies. The genetic apparatus of somatic cells clearly and actively reacts to numerous environmental influences on the organism. It allows to detect early changes in the functional systems of the organism, when there are no visible (phenotypic) manifestations of these changes yet. It induces mutations detectable in Ames test, chromosomal aberrations, sister chromatid exchanges, etc. (Hobbs et al., 2015; Wagner et al., 2012; Watanabe and Kamiguchi, 2001; Nechaykina et al., 2022; Lovinskaya et al., 2017). The mutagenic effect of unsymmetrical dimethylhydrazine (NDMH) on rats of different age groups at acute and subacute exposure has been shown to depend on the dose and duration of exposure.

The organ specificity of genotoxic action of NDMA has been revealed. Kidney, liver, and germ cells turned out to be the most sensitive organs (Lovinskaya et al., 2017; Oliveira et al., 2020). However, in vivo studies on animals are few and, as a rule, have been conducted in laboratory conditions (Lovinskaya et al., 2017; Oliveira et al., 2020). One of the main indicators of the influence of negative factors that can generate significant changes in the state of ecosystems and the organism as a whole is cytogenetic homeostasis. In wild animals, it can be characterized using the micronucleus test (Şekeroğlu et al., 2013; Abhishek et al., 2014). Additional information about the negative processes occurring in response to the impact of stressor environmental factors can be obtained by analyzing other nuclear disorders. Recently, the evaluation of the complex of nuclear disorders is called cytomic analysis.

Cytogenetic analysis showed a statistically significant increase in the frequency of micronuclei and cytological disorders in erythrocytes of peripheral blood of indicator animals living in the emergency area, which indicate the existing degenerative processes in the organism, which may be due to various reasons, including those of environmental nature and may be a consequence of persisting toxic effects inducing cyto- and genotoxicity.

It is common that under different physiologic states of the organism, specific forms and spectrum of cytologic disorders occur. In many cases, these changes accompany compensatory processes occurring in tissues, for example, under functional overload, poisoning, or not good ecological conditions (Schmid, 1975; Mitkovska et al., 2012; Anbumani & Mohankumar, 2015).

Likewise, it is worth noting that cyto-pathologic changes in erythrocyte structure in animals of various classes occur with different frequencies and have a distinctive spectrum and their species specificity as it has been observed in marine and other fishes (Dar et al., 2016; Guilherme et al., 2008; Schaumburg et al., 2012; Strunjak-Perovic et al., 2009).

According to the experience of our current and previous studies, we can mention that the highest frequency and a vast range of cytological disorders in animals with nuclear erythrocytes are typical for fish, birds, and reptiles in descending sequence while it is

least prevalent in amphibians. At the same time, the frequency of cytological disorders is not related to the frequency of registered micronuclei, and can be both the result of anthropogenic factors and species or individual peculiarity of the studied animal.

Based on the analysis of a wide-range morphophenetic and cytogenetic studies of genus *Eremias* and rodents it should be noted their different susceptibility and level of distinction from the control. Morphological tests of rodents did not reveal any differences from species norms whereas cytogenetic analysis found an increased frequency of micronuclei.

Conclusions

In order to determine the long-term effects from the "Dnepr LV" accident as well as from the liquidation actions implemented in the emergency area it has been accomplished a comprehensive study of the territory and the state of the fauna of reptiles and mammals. The study was carried out taking into account a retrospective analysis of the situation in the area of the launch vehicle crash and a mixed type of impact. To identify possible long-term environmental consequences and assess the condition of animals it was used an approach enabling to trace the progress of implementing each action according to the following stepwise scheme:

- Toxicological indicators of the habitat;
- Species diversity of animals and its conservation;
- Morphophenetic characteristics and indicators of fluctuating asymmetry of the indicator group;
- Genetic status of animals of indicator groups.

Testing of soil samples taken at the site of the Dnepr rocket launcher accident and surrounding areas using two test systems showed a statistically significant increased level of genotoxicity of samples taken at the accident site and adjacent areas (d≈250 m) compared to internal and external controls.

As a result of the study, the species composition of indicator species of animals was clarified among representatives of indicator groups living in the emergency territory, which did not reveal any deviations and corresponds to the natural climatic and seasonal conditions of stay.

The set of data, including the ecological and morph-functional characteristics of animals living in the area of the Dnepr LV accident, was without significant distinctive features and corresponded to the limits of variation in the indicators of animals living in Kazakhstan. However, for the average and lined *Eremias* lizards, a slightly higher level of fluctuating asymmetry was shown, which may indicate a not very stable development of these populations during the periods of collecting material at the site of the launch vehicle accident. The results obtained generally indicate that the state of the studied animal populations in the area affected by the accidental fall of the Dnepr launch vehicle is currently satisfactory.

Despite the absence of morph-functional changes in the examined animals, an assessment of the genetic status of indicator animals captured in the studied area revealed a statistically significant increase in the frequency of micronuclei and cytological disorders in peripheral blood erythrocytes.

Thus, the use of a stepwise analysis scheme in assessing the state of the herpetofauna and mammals based on the determination of a number of informative indicators of indicator group animals allows us to draw a conclusion about the presence of long-term consequences of the impact of the Dnepr launch vehicle accident on the herpetofauna and mammals. Thus, the results of the study showed that the presence of an increased level of soil genotoxicity and changes from the norm in individual morphophenetic and micronuclear parameters did not turn out to be critical for the existence of this group of vertebrate animals.

This is explained by the fact that the studied populations of reptiles and mammals (rodents) probably have a fairly high adaptive potential at the physiological level and the presence of compensatory mechanisms. This is confirmed by the totality of information obtained on normal occurrence rates, preservation of species diversity, reproductive success, and basic morphometric characteristics, which allows us to speak about the relatively favorable state of the herpetofauna in the area of the Dnepr launch vehicle accident.

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Data availability The original contributions presented in the study are included in the article; further inquiries can be directed to the corresponding author.

Declarations

Ethics approval Every participant (Oksana Cherednichenko, Marina Chirikova, Igor Magda, Oleg Lopatin, Serikbay Nuraliyev, Anastassiya Pilyugina, and Dinara Azizbekova) has agreed to participate in this research. All authors have read, understood, and have complied as applicable with the statement on "Ethical responsibilities of Authors" as found in the Instructions for Authors.

Competing interests The authors declare no competing interests.

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